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ULTRASTRUCTURAL EVIDENCE THAT GASTROPODS SWALLOW SHELL RASPED DURING HOLE BORING

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Several investigators have demonstrated by light microscopy that shell scraped from the borehole during penetration of valves of prey by three species of muricid and naticid gastropods is swallowed and eliminated in the feces. Graham (1941) discovered that the stomach of specimens of *Nucella lapillus* which were boring the valves of *Mytilus edulis* contained minute curved flakes of crystalline material which dissolved with effervescence on the addition of hydrochloric acid, whereas these were absent from the stomach of snails not boring (see also Fretter and Graham, 1962). Graham (1953) noted further that the calcareous material in the stomach was embedded in a thick mucous material probably provided by the salivary glands and possibly also by the accessory salivary glands. Ziegelmeier (1954) was able to see white shell material passing down the esophagus of actively boring *Natica nitida*, and during subsequent defecation observed white pellets composed mainly of shell fragments in the feces. Carriker and Van Zandt (1972b), employing a contact microhydrophone to identify rasping periods, removed *Urosalpinx cinerea* from the valves of *Mya arenaria* at the end of the rasping period and found white shell raspings bound by a mucus-like substance in string-shaped pellets in the stomach. Pellets were birefringent under polarized light, became red when stained with alizarin sodium monosulfonate, and consisted of distinct solid particles amid soft mushy shell material. Fecal strings collected in dishes from snails which were boring small *Crassostrea virginica* also contained conspicuous white shell raspings which were likewise identified as calcium carbonate. Shell fragments appeared to be dissolved only slightly, but this could not be determined with certainty with the light microscope.

The purpose of this paper is to report observations on the ultrastructure of shell material rasped by *Urosalpinx cinerea follyensis* Baker from boreholes in the valves of *Mytilus edulis* Linné and transported normally to the stomach through the buccal cavity and esophagus.

Materials and Methods

Snails used for the study came from Wachapreague, Virigina. They were maintained in the laboratory in running sea water (approximately 30–32‰, at room temperature) and bored and fed actively on the bivalves *Mytilus edulis* and *Crassostrea virginica* for several weeks prior to use in these observatons.

Duration of the period of chemical activity by the accessory boring organ and of rasping by the radula were determined in a valve model (Carriker and Van Zandt, 1972b) as follows. After a snail had bored about half way through the valve of a live *Mytilus edulis*, the free valve and flesh of the mussel were gently removed under water and the remaining half-shell boring-snail preparation was positioned, inner surface of the valve facing up and the snail suspended underneath, on a platform in slowly running sea water under a binocular microscope. The inner

nacreous layer of the shell of *M. edulis* is translucent, and activity of the proboscis, radula, and accessory boring organ were visible as excavation of the borehole by the snail neared the inner surface of the valve. It was thus possible to determine accurately the duration of chemical and rasping periods.

After several preliminary anatomical dissections to locate shell raspings accurately in the stomach, three snails which were boring the valves of live *Mytilus edulis* were selected, and the snail-valve models were prepared. The mussels were 6 cm long.

The height in millimeters of each snail, duration in minutes of the period of chemical activity by the accessory boring organ prior to the rasping period, and the number of rasps during the rasping period just before the snail was removed for dissection were as follows: specimen 1, 35 mm, 55 min, 12 rasps; specimen 2, 31 mm, 11 min, 25 rasps; and specimen 3, 35 mm, 21 min, 40 rasps. Diameter of boreholes emerging on the interior surface of the valves was about 0.8 to 1 mm, and that on the exterior of the valves was approximately 1.2 mm.

As soon as the first snail was removed from the snail-valve preparation, its shell was immediately cracked, the soft parts removed and pinned on a small dissecting pan under sea water; the stomach was opened with fine dissecting instruments and the pellet of shell raspings was removed with a fine bulb pipette. The stomach of muricid gastropods is a simple sac (Fretter and Graham, 1962), so it was easy to locate the pellet precisely. About 5 min elapsed from the time the snail was removed from the snail-valve preparation and the time the pellet was taken from the stomach.

The pellet was spread evenly over the surface of a polished brass scanning electron microscope (SEM) stub and dried at room temperature. The following day, the specimen was rinsed briefly with distilled water to dissolve salt left by sea water, quickly dried again in air, and coated in vacuum with platinum-palladium. Examination of the specimen in the scanning electron microscope (JEOLCO JSM–U3) showed that the material was charging badly, so it was dried further in an oven at 105° C for six days. Because the mucoid coating on the exterior of the pellet obscured the contents, the mass was then opened with a fine needle point, and was shadowed again with platinum-palladium. There was still some charging, so a final layer of gold was applied in vacuum and this eliminated most of the charging. Pellets from the other snails were treated in the same way.

Ultrastructure of the normal valves of *Mytilus edulis* was examined as follows for comparison with the shell fragments swallowed by *Urosalpinx cinerea*. The valves of five *M. edulis*, 5 to 6 cm long, were removed from the soft parts, cleaned in water, dried, and fractured into small pieces with a hammer on a hard surface. Under a binocular microscope pieces were selected from the central area of the valves whose fractured surfaces exposed cross, side, and oblique sections of prisms from the prismatic region, and cross and oblique sections of lamellae from the nacreous region. Some of the fractured pieces were mounted on SEM stubs, coated with gold, and examined directly in a scanning electron microscope. Others were treated chemically as follows to reveal the outlines of shell units more clearly than was possible without the treatment: (a) immersion in 80% (by volume) ethylenediamine for about 20 hr at room temperature and washed with water, (see Figs. 8, 9, 11); and (b) immersion in 5.25% sodium hypochlorite (commercial

Clorox) for 2.75 hr with intermittent sonication for a total of 65 min, followed by retention and washing with water on a Millipore filter (0.45 μm pore size) with 5 to 10 lb. pressure from the vacuum pump (see Fig. 12). Specimens (a) and (b) were dried, mounted on SEM stubs, the latter still on a piece of Millipore filter, coated with gold, and examined in a scanning electron microscope. Sodium hypochlorite (Mutvei, 1970, 1972) and ethylenediamine (Zapanta and Trautz, 1961) selectively dissolved the organic matrix of the shell pieces, but did not noticeably alter the appearance of the mineral crystals.

Results

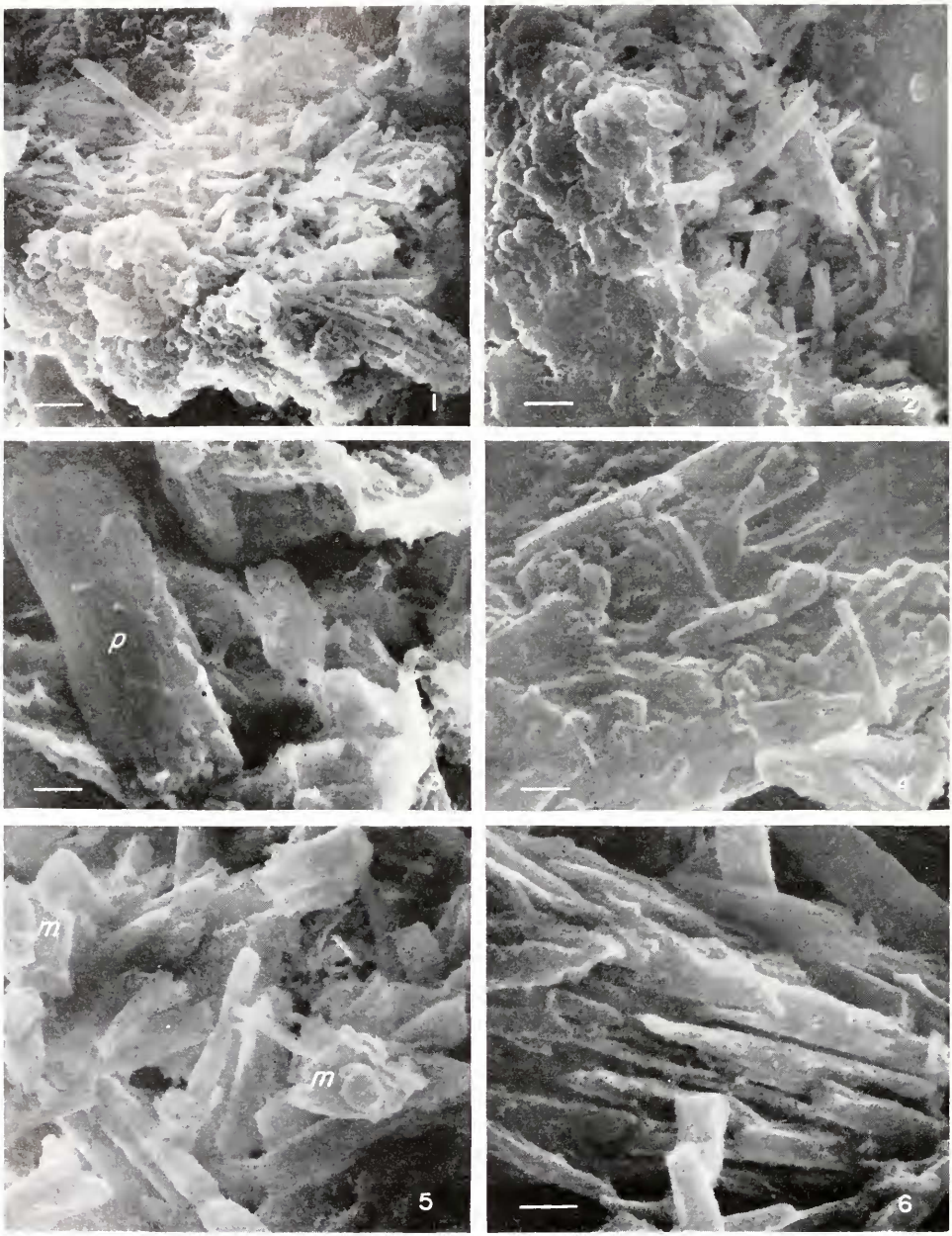
Scanning electron microscopy clearly confirmed the presence of shell fragments in the stomach of *Urosalpinx cinerea*. When large amounts of shell were removed during a rasping period, shell fragments tended to be clustered within an envelope of mucoid material and were not visible until the pellet was fractured open (Figs. 1, 2). When less shell material was scraped off by the radula, fragments tended to be embedded in the mucoid material and were more difficult to see even after the pellet was fractured (Fig. 4).

Two kinds of mineral shell units, prisms and lamellae, (Zottoli and Carriker, 1974) characteristic of the shell of *Mytilus edulis* were identified in the scanning electron micrographs. Prisms, the long slender pencil-shaped calcitic structures (Figs. 1–6) were abundant; whereas groups of the thin wafer-like aragonitic lamellae (also called tablets by some investigators) were seen only occasionally (Fig. 5).

Length of prisms in the pellets varied from pieces 15 to 18 μm (Figs. 1, 6) to small fragments (Figs. 4, 5). Bundles of prisms, still bound together by organic matrix, were also seen occasionally (Fig. 3).

As a basis for comparison of the size of prismatic shell fragments in the stomach of *Urosalpinx cinerea* with the size of prisms in undisturbed shells, the cross sectional dimensions (height and width) of 66 prisms in fractures of shell from three *Mytilus edulis* approximately 5 to 6 cm long (Fig. 13) and magnified 6000 to 10,000 times in scaning electron micrographs were measured. Sections of shell were selected in which fractures occurred at right angles to the long axes of the prisms to avoid angular distortion in the measurements. An oblique view—not at right angles to the long axis—of a fracture of prisms is illustrated in Figure 7. The cross sectional anvil shape of the prisms is characteristic. A low and a higher magnification of isolated single prisms are seen in Figures 8 and 11, respectively. The height of prisms (Fig. 13) varied from 0.6 to 2.0 μm and the width ranged from 1.1 to 2.8 μm; the arithmetic average height of prisms was 1.2 μm and the arithmetic average width was 2.1 μm. These dimensions compare favorably with those reported by Travis and Gonsalves (1969) for cross sections of prisms of the same species in electron microscopic thin sections. Several isolated single prisms (for example, Fig. 8) ranged maximally from 56 to 65 μm in length, but it is difficult to say whether this was the actual length of the prisms, or whether they were broken in the process of isolation.

The maximum "width" of the 212 prismatic fragments measured in the stomach of *Urosalpinx cinerea* (Figs. 1, 2, 4, and other micrographs not included here) ranged from 0.25 to 2.4 μm (Fig. 14). These sizes fall within the height-



FIGURES 1–6. Scanning electron micrographs of pellets of shell raspings and mucoid material removed from the stomach of *Urosalpinx cinerea* immediately after the rasping period. FIGURE 1: half a pellet opened to show interior filled with fragments of shell prisms. The dried nodular mucoid coat is on the exterior to the left; specimen 1; scale bar squals 5 μm. Figure 2: a second view of the same pellet emhasizing the mucoid coat and outlines of shell prisms; specimen 1; scale bar equals 5 μm. Figure 3: an intact bundle (p) of many shell

width dimensions of prisms measured in unrasped shell (Fig. 13), allowing some reduction in size of prisms from dissolution by the secretion of the accessory boring organ. The tapering ends and spaces among prisms in Figure 6 demonstrate the extent of dissolution that took place in the borehole prior to rasping and probably to a slight extent during passage of prisms surrounded by secretion down the esophagus to the stomach. Figures 1–5 suggest the extent of cross-sectional breakage of prisms that occurred during the rasping period (compare with Fig. 8). The "width" could represent either the height or the width of prisms, depending on their orientation in the specimens examined.

Noticeable dissolution of the organic matrix, and to some extent of the mineral portion, of prisms was evident (Fig. 6). This resulted in tapering ends and conspicuous spaces among the prisms, in marked contrast to the bundles of prisms which were uprooted before they were affected by the secretion (Fig. 3).

As a basis for comparison of the thickness of lamellar shell fragments in the stomach of *Urosalpinx cinerea* with the thickness of lamellae in undisturbed shell, the thickness (or height) of 164 lamellae in fractures of pieces of valves from three different *Mytilus edulis* approximately 5 to 6 cm long in scanning electron micrographs magnified 8000 times were measured. Sections were selected in which fractures occurred at right angles to the long axes of the lamellae (as in Fig. 10). The polygonal form of lamellae is illustrated in Figues 9 and 12. A few isolated lamellae are visible from the side in the mid-left of Figure 12, much as they appear in the stomach pellets (Fig. 5) of *U. cinerea*. Thickness of lamellae in undisturbed shell of three different individuals of *M. edulis* ranged from 0.25 to 2.0 μm (Fig. 15). Study of additional fractures of nacreous shell disclosed that the thickness of lamellae varied widely from individual to individual mussel, and that lamellae formed at the juncture of myostracum and naceous and prismatic strata were as much as one-half to one-third thinner than elsewhere in the shell. The bimodal curve in Figure 15 is indicative of the differences observed in the size of lamellae from the center of nacreous strata (not adjacent to myostracum) in three different individuals.

Only three lamellae ranging in thickness from 0.2 to 0.3 μm were seen among the shell fragments swallowed by *Urosalpinx cinerea* in Figure 5 (compare with Fig. 15).

From information now available, it is possible to estimate the quantity of shell excavated by the radula and swallowed during hole boring. Carriker (1969) estimated that the total surface area of the bottom of a borehole 1.47 mm in diameter rasped during a rasping period ranged from 1/10 to 1/5. Depth of rasp marks varies widely with the hardness of the shell (Carriker, 1969). As a basis for calculation let us assume an average depth of each rasp mark of 6 μm in the shell of *Mytilus edulis* (see Fig. 13, in Carriker, Schaadt, and Peters, 1974) ; an average surface area at the bottom of the borehole of 0.785 mm^2 (based on an average

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prisms dislodged by the radula from beneath the zone of dissolution in the borehole and showing no, or little, evidence of dissolution; specimen 3; scale bar equals 2 μm. Figure 4: interior of a portion of a pellet with few shell prisms embedded in mucoid material; specimen 3; scale bar equals 1 μm. Figure 5: two prominent clusters of shell lamellae (m) among a rubble of prisms; specimen 1; scale bar equals 2 μm. Figure 6: organic matrix and part of the calcareous cores of shell prisms noticeably dissolved; specimen 1; scale bar equals 1.5 μm.


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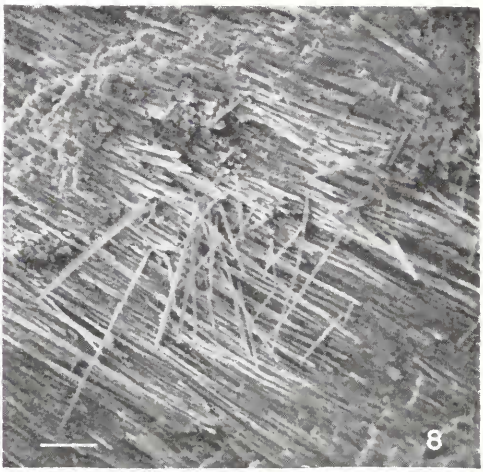
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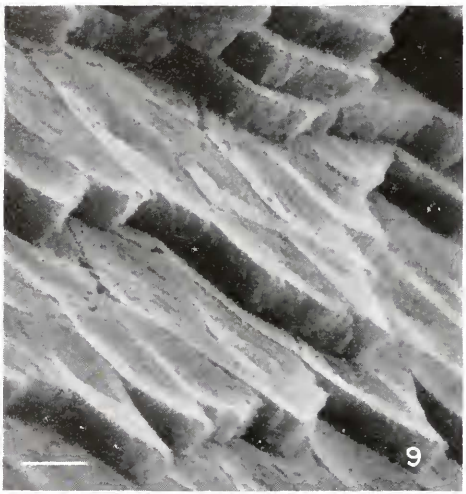
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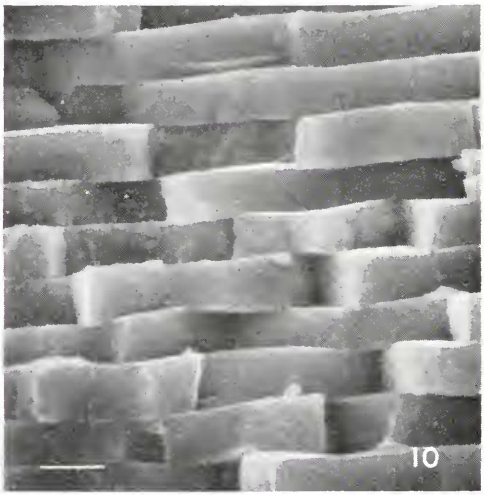
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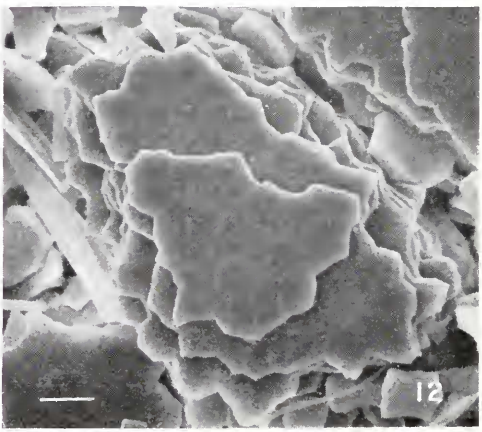
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diameter of 1 mm since the borehole tapers from exterior to interior); a total of 150 rasping periods for penetrating an oyster valve 1.0 mm thick (Carriker and Van Zandt, 1927b); and removal of shell from 15% of the surface of the borehole at each rasping period. Thus of a total of 0.785 mm^3 of shell removed from the borehole by combined chemical and mechanical activity, 0.106 mm^3 was rasped out by the radula. It follows then that swallowing accounted for roughly only 14% of the shell in the borehole. These calculations are based on gross estimates, particularly those of the amount of shell actually removed by each rasping stroke and the number of strokes per rasping period, and thus must be used with caution.

Discussion

The study provided ultrastructural evidence for the first time that *Urosalpinx cinerea* swallows shell rasped from the borehole during penetration of prey.

The predominance of prisms in the pellets indicates that snails had rasped through the outer prismatic region of the shell of *Mytilus edulis* when sacrificed for analysis of stomach contents. The scarcity of lamellae and their minute size suggest that the snails had just commenced rasping in the nacreous stratum of the shell.

The chemical composition of the mucoid envelope surrounding shell raspings in the stomach is unknown. The mucoid material may consist of a mixture of secretions from the accessory boring organ wiped from the bottom of the borehole during rasping, of mucus secreted by buccal glands, esophageal mucous glands, and possibly also of secretion from the salivary glands and the gland of Leiblein. Graham (1953) and Fretter and Graham (1962) concluded that the accessory salivary glands, as well as the accessory boring organ, seem to be necessary for hole boring as these organs are present in the shell boring muricacean stenoglossan gastropods, but are absent in the next two super-families, the Buccinacea and Volutacea. Precisely what role the accessory salivary glands play in shell excavation is unclear, but that they are involved appears a logical deduction from the position of their external aperture in the front midventral portion of the buccal cavity (Graham, 1953). At first glance absence of accessory salivary glands from predatory, shell boring, naticacean gastropods seems to negate this interpretation. However, there is a possibility that the accessory salivary glands may be functionally replaced by the collar of mucus-like secreting cells which surround naticid accessory boring organs. This provocative area of research awaits the histochemist and physiologist.

Figures 7–12. Scanning electron micrographs of the structure of normal shells of adult *Mytilus edulis*. Figure 7: ends and sides of prisms in a cross sectional fracture of shell, treated with phosphate buffer at pH 7; scale bar equals 2 μm. Figure 8: prismatic shell treated with ethylenediamine to free the surface prisms from the organic matrix; scale bar equals 20 μm. Figure 9: oblique view of terraces of nacre treated with sodium hypochlorite to expose boundaries of individual lamellae; scale bar equals 2 μm. Figure 10: fracture of nacre exposing cross sections (heights) of lamellae, treated with sodium hypochlorite; scale bar equals 2 μm. Figure 11: prisms freed from the organic matrix by immersion in ethylenediamine; scale bar equals 2 μm. Figure 12: clusters and single lamellae freed from nacre by treatment with sodium hypochlorite and sonication; scale bar equals 5 μm.

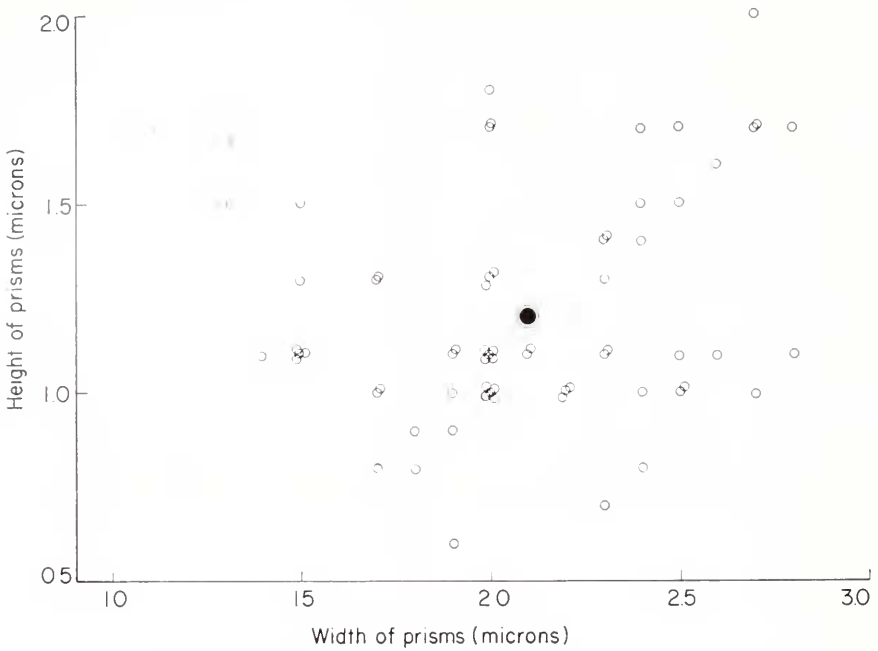

FIGURE 13. Plot of cross sectional dimensions of prisms in fractured shell of adult *Mytilus edulis;* solid circle represents arithmetic average height and width of prisms.

Most of the dissolution of shell probably takes place on the surface of the borehole prior to the rasping period (Carriker, 1969). The characteristic initial dissolution of cementing organic matrix of shell units (Carriker, 1969; Carriker,

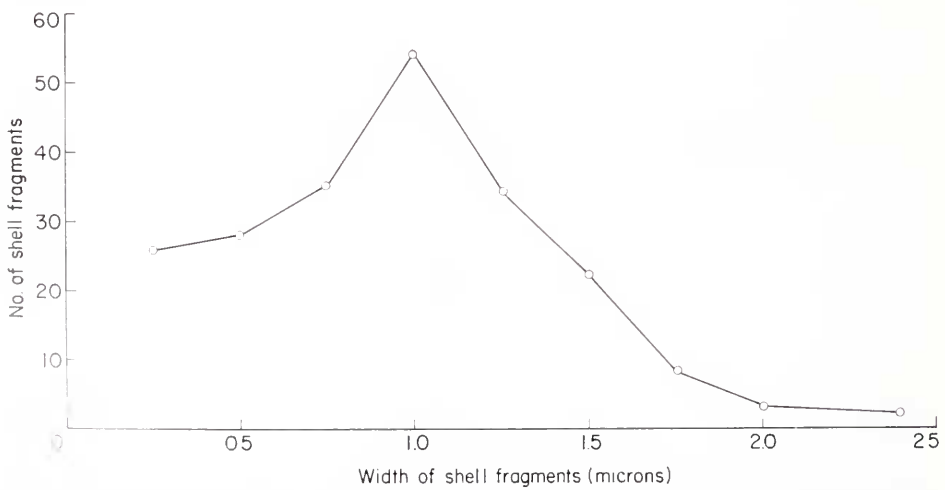

FIGURE 14. Plot of "width" of prismatic fragments taken from stomach of *Urosalpinx cinerea.*

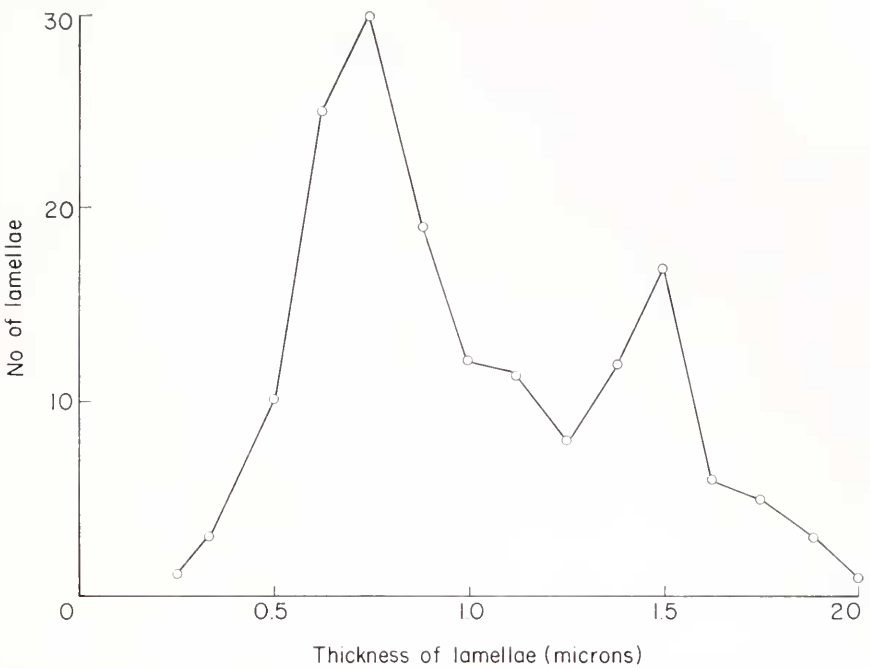

FIGURE 15. Plot of thickness of lamellae in fractured shell of adult *Mytilus edulis*.

Schaadt, and Peters, 1974) creates spaces among them, thereby weakening the shell sufficiently for the radula to dislodge and remove the units. A similar type of dissolution of the shell of *Mytilus edulis* is caused by secretions from the shell burrowing polychaete, *Polydora websteri* (Zottoli and Carriker, 1974). If prisms occur parrallel to the surface of the borehole (much as in Fig. 5d, Zottoli and Carriker, 1974), the radula breaks out long fragments of shell (Fig. 1, this paper); on the other hand, if prisms occur more or less at right angles to the surface of the borehole (much as in Fig. 13, Carriker, Schaadt, and Peters, 1974), the radula tends to cut out mostly short pieces of shell units (Fig. 5, this paper).

The mucoid material which blankets shell fragments on their journey from the buccal cavity to the stomach and out the intestine probably insulates them against further corrosion from acid juices in the stomach. More importantly, however, the mucoid envelope must also serve to insulate sharp edges of shell fragments which might otherwise lacerate the delicate ciliated epithelium of the alimentary canal. Since the mucoid material undoubtedly also contains a large proportion of viscid secretion from the accessory boring organ, it would appear that a secondary function of the secretion from this gland is to contribute to clothing of sharp shell fragments on their way down the digestive tract.

It is inviting to speculate whether hole boring contributes calcium carbonate to the calcium anabolism of boring snails. Observations reported in this paper, however, suggest that very little dissolution of the shell occurs in the stomach. The question could be settled by incorporating a calcium isotope in wafers of ground

shell rasped by snails in devices patterned after the valve model of Carriker and Van Zandt (1972b). The isotope could then be traced through the organ systems of the snail. Further support for the conjecture that the snail does not utilize its shell chips to any extent in supplementing its calcium needs comes from the fact that shell pellets remain only briefly in the stomach.

Why shell rasped from the borehole is swallowed rather than pushed out by the propodium in its sweep across the surface of the borehole after each rasping period (Carriker and Van Zandt, 1972b) may be explained on morphological grounds. Simply stated, the effective stroke of the radula over the substratum is from front to back (Carriker, Schaadt, and Peters, 1974), and the load gathered on each stroke is dumped onto the esophageal valve and by suction from the anterior esophagus is carried immediately into the esophagus. Furthermore, the valve of Leiblein present in the esophagus of snails with long proboscides, like those of the Muricacea and Buccinacea, is thought to prevent regurgitation of food from the more posterior parts of the gut during elongation of the proboscis (Fretter and Graham, 1962), an arrangement which would tend to deter necessary reversal of flow in the anterior alimentary canal required for oral discharge of shell raspings.

Although the accessory boring organ determines the shape and size of the borehole through dissolution of shell (Carriker and Van Zandt, 1972a), no one has calculated how much dissolved calcium or other components of shell are absorbed into the body of the snail through the accessory boring organ during the process of shell boring. It is likely that a substantial amount of the dissolved shell mixes with the secretion of the accessory boring organ and, with shell chips broken off by the radula, finds its way down the alimentary canal.

This study graphically demonstrated the extraordinary capacity of boring gastropods to dismantle the intricately architectured mineralized valves of prey in search for food. The process plainly combines mechanical-chemical functions (Carriker and Van Zandt, 1972b), and effective removal of shell is impossible by radular or chemical action alone (Carriker, Person, Libbin, and Van Zandt, 1972; Carriker and Van Zandt, 1972b). Undoubtedly, rasping came first in the evolution of the group because the radula appeared early as a tool in food procurement. The next step, of rasping the shell of prey as patches of shell dissolving glandular tissue evolved to facilitate penetration, would be a logical one—and with it the act of swallowing shell chips simply as an available, functional, and economical avenue for their discharge!

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Summary

1. Observations are reported on the ultrastructure of shell material rasped by *Urosalpinx cinerea follyensis* Baker from boreholes in the valves of *Mytilus edulis* Linné and transported normally to the stomach through the buccal cavity and esophagus. Duration of the period of chemical activity by the accessory boring organ and rasping by the radula were determined with a valve model. Pellets of shell raspings were removed from the stomach and, after fracturing to reveal the interior, and coating with metal, were studied with the scanning electron microscope. Shell raspings were compared with prisms and lamellae in fracture surfaces of normal shell of *M. edulis* and shell etched with ethylenediamine and sodium hypochlorite to reveal the form of shell units clearly.

2. The study provided ultrastructural evidence for the first time that *Urosalpinx cinerea* swallows shell rasped from the borehole during penetration of prey. Both prisms and lamellae were identified in the pellets removed from the stomach. Noticeable dissolution of the organic matrix, and to some extent also of the mineral portion, of prisms was evident, features which facilitate removal of shell by the snail during rasping.

3. If the long axis of prisms occurs parallel to the surface of the borehole, the radula tends to rasp out long fragments of shell; if prisms are placed at right angles to the surface, the radula breaks prisms into small pieces.

4. The envelope of mucoid material which coats pellets undoubtedly reduces, or prevents, laceration of the epithelium of the alimentary canal as shell fragments pass down the tract.

5. A gross approximation of the percentage of shell in the borehole which is rasped and swallowed during the process of hole boring is 14%.

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